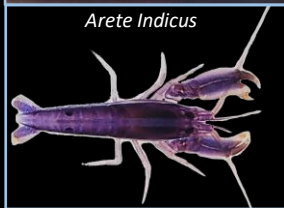




## Introduction and aim of the study

- **Symbiotic associations** are omnipresent in marine ecosystems with some symbionts are **deeply dependent from their host and their chemical environment**. They may die if isolated from their host<sup>1,2,3</sup>.
- Two symbiotic shrimps, *Tuleariocaris holthuisi* and *Arete indicus* are commonly found as dependent ectosymbionts on their sea urchin host *Echinometra mathaei* on the Great Reef of Toliara, Madagascar (Fig. 1 and 2)<sup>1,2</sup>. *T. holthuisi* presents a severe “**host separation syndrome**” when isolated from its host which results into a **severe discoloration of their exoskeleton and a lower survival rate**<sup>1,2</sup>. Specific host pigments, called **spinochromes**, were shown to be essential to the survival rate of *T. holthuisi*<sup>1,2</sup>.



**Figure 1:** *Echinometra mathaei* and its symbionts, *Tuleariocaris holthuisi* and *Arete indicus*.



**Figure 2:** The Great Reef of Toliara, Madagascar (Google Earth)

**A.** Symbionts present on their host, **B.** Symbionts isolated from their host **C.** Symbionts isolated from their hosts in sea water containing spinochromes extracted from the host (Fig. 2).

The present study aims at evaluating the stress induced by the host separation syndrome using a transcriptomic approach (Paired-end Illumina HiSeq technology) on *T. holthuisi* under three different conditions: (A.) symbionts present on their host, (B.) symbionts isolated from their host (C.) symbionts isolated from their hosts in sea water containing spinochromes extracted from the host (Fig. 2).

**Figure 2:** Experimental design of the study testing 3 different conditions on *T. holthuisi*. A. Control ; B. Isolated and C. Isolated in conditioned water with spinochromes.

## Results and discussion

- **A total of 217,832 assembled unigenes, with a N50 of 2,061 bp.**

<b>Vs</b>	<b>30,025 DEGs</b> (Differentially Expressed Genes)	<b>13,588 DEGs</b>
	<b>15,557 DEGs</b>	<b>10,473 DEGs</b>

### Cuticle and chitin proteins

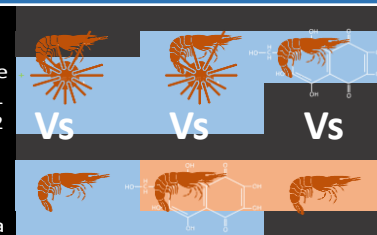
- **16 cuticle and chitin**
- **2 Basic proline rich**
- **1 Crustacyanin**

- Up-regulation of unigenes related to the cuticle and chitin protein production in case of isolation for *T. holthuisi*

- **Host separation impacts shell coloration and molting (Figs.4; 5)**

### Heat Shock Proteins

- Heat shock protein 10
- Heat shock protein 21
- Heat shock protein 67B1-like
- Heat shock protein 68-like 1
- Heat shock protein 68-like 2
- Heat shock protein 70
- Heat shock protein 75 kDa
- Heat shock protein 90 beta
- Heat shock protein 90, alpha



- In pairwise comparison with the control condition, the two treatments showed differential expression of Heat Shock Proteins.

- **Heat Shock Proteins may be a good indicator for host separation stress independently of the chemical environment.**



**Figure 4:** *Synalpheus stimpsonii*, a symbiotic shrimp that loses its colors when isolated from its crinoid host.



**Figure 5:** *Lissocarcinus orbicularis* seems to molt more often when isolated from its holothurian host.

## Conclusions

This work represents the first report of the utilization of the NGS techniques for the study of the host-separation syndrome. Based on the comparison of gene expressions, sequences related to the cuticle and chitin protein production and Heat Shock Proteins seem to be differentially expressed depending on the host-isolation syndrome.

## Funding

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## References

- <sup>1</sup>Brasseur, L et al. (2018). *Echinometra mathaei* and its ectocommensal shrimps: the role of sea urchin spinochrome pigments in the symbiotic association. <sup>2</sup>Lourtie, A et al. (In prep) Host-separation syndrome in two ectocommensal shrimps from the South-West of Madagascar: a transcriptomic approach. <sup>3</sup> Caulier, G et al. (In prep). Crinoid anthraquinones as kairomones allowing host selection for the symbiotic snapping shrimp *Synalpheus stimpsonii*